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Risk Factors and Molecular Analysis of Community Methicillin-Resistant *Staphylococcus aureus* Carriage

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A total of 1,838 subjects from the community and 393 subjects from health care-related facilities in Taiwan were evaluated for the prevalence of nasal *Staphylococcus aureus* colonization and to identify risk factors associated with *S. aureus* and methicillin-resistant *S. aureus* (MRSA) colonization. Among the community subjects, 3.5% had nasal MRSA colonization. Subjects from health care-related facilities had a lower *S. aureus* colonization rate (19.1%) than community subjects (25.2%) but had a significantly higher rate of colonization with MRSA (7.63%). Age ($P < 0.001$) was a significant risk factor for *S. aureus* colonization, with subjects under age 20 years or between 71 and 80 years showing higher rates of colonization. Recent gastrointestinal disease ($P = 0.011$) and hospital admission ($P = 0.026$) were risk factors for nasal MRSA colonization. Comparison of hospital MRSA isolates with the colonization strains by staphylococcal cassette chromosome *mec* (SCC*mec*) gene typing and pulsed-field gel electrophoresis (PFGE) typing revealed that most MRSA strains carried in the community were SCC*mec* type IV and that most clinical hospital isolates were type III, while health care facility-related carriage isolates were mainly SCC*mec* type III and type IV. Two new variant SCC*mec* types were identified. Six clusters of PFGE patterns were distinguished: two mainly comprised health care facility-related MRSA strains, three mainly comprised community MRSA strains, and one comprised mixed community and health care facility-related MRSA strains. In conclusion, a high prevalence of MRSA colonization was observed among people with no relationship to the hospital setting. The high level of multiple-drug resistance among community MRSA strains in association with the previously reported excessive use of antibiotics in Taiwan highlights the importance of the problem of antibiotic selective pressure. Our results indicate that both the clonal spread of MRSA and the transmission of hospital isolates contribute to the high MRSA burden in the community.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat to hospitalized patients globally and now represents a challenge for public health, as community-acquired infections appear to be on the increase (18) in both adults and children in various regions and countries, including North America (10, 16, 18, 27, 35), Australia (23), Saudi Arabia (24), Finland (34), New Zealand (32), the United Kingdom (37), and Taiwan (12, 41).

It was estimated that MRSA strains accounted for 84% of hospital-acquired *S. aureus* isolates and 45% of non-hospital-acquired *S. aureus* isolates in Taiwan in 1998 (12). Recent reports from Taiwan that isolates causing 27.8% of community-acquired *S. aureus* infections in children were resistant to oxacillin, that 35.4% of community-acquired MRSA isolates were from children without any predisposing risk factors (41), and that severe diseases such as infective endocarditis have resulted from community-acquired MRSA (19) should alert medical professionals and the community alike to the need for the country to face the problem of MRSA within the community.

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Community-acquired MRSA (C-MRSA) in the United States and Australia had a staphylococcal cassette chromosome *mec* (SCC*mec*) type (i.e., type IV) (30) different from those of the health care setting-associated MRSA (H-MRSA) strains, whose SCC*mec* types were mainly types I to III (11, 14). Although a previous study revealed that SCC*mec* types III and IIIA were the main types of clinical MRSA strains in Taiwan and China (1), the SCC*mec* types of C-MRSA strains in Taiwan have not been studied. It is of interest to determine whether SCC*mec* type IV, which represents a particular clone that is disseminated in the community, is prevalent in Taiwan, where there is a very high prevalence MRSA and a high extent of multidrug resistance among hospital MRSA isolates (8, 41).

Nasal *S. aureus* colonization has been shown to be a risk factor for community-acquired and nosocomial infections (7, 17, 40). Previous studies of nasal MRSA colonization found that the rates of colonization with MRSA among community residents without any predisposing factors were less than 1% in New York City; San Francisco, Calif.; and Portugal (6, 33). Taiwan has high percentages of MRSA in hospitals and a high extent of antibiotic use in the community (20) and is under an increasing threat from community-based MRSA infections (12, 19, 41). This study evaluated the burden of *S. aureus* resistance in the community, the potential risk factors for nasal *S. aureus*

and MRSA colonization, and the molecular characteristics of MRSA isolates from the community and clinical settings.

MATERIALS AND METHODS

Study duration and population. The study was performed during the 7-month period from 1 April to 31 October 2001, with residents of Pingtung County, Taiwan, serving as subjects. The study population comprised 1,838 community residents. All residents of two villages and volunteer residents at another two villages and four sites in Pingtong City were included. The individuals from the four sites in Pingtong City included all students at a kindergarten, all students at an elementary school, and students from three classes of every grade of a junior high school and a senior high school. The selection of these schools from all schools in Pingtong was based on support for the surveillance study by the schools' principals. The three classes of each grade of the junior and senior high schools were randomly selected. Other study participants were associated with health care facilities, including all 100 subjects living in a nursing home, all 85 hemodialysis patients treated in an outpatient setting at hemodialysis unit, 69 volunteers from acute-care wards, 120 patients hospitalized in acute-care wards, and 139 volunteers among a total of 200 health care workers from Pingtung Hospital. This hospital is one of three accredited regional hospitals in Pingtong County. A total of 2,231 subjects underwent the same nasal specimen acquisition procedure and completed the questionnaire as part of the study. MRSA isolates from clinical specimens of hospitalized patients in Pingtung Hospital were concurrently collected during the study period for molecular typing.

Microbiological study. All study participants underwent swabbing of the anterior 1.5 cm of the nasal vestibule of both nares with a sterile swab (CultureSwab Transport System; Difco, Detroit, Mich.). The swab specimen was streaked onto two mannitol salt agar plates (Difco, Sparks, Md.), one of which was supplemented with oxacillin (4 μg/ml). These inoculated plates were incubated at 37°C for 48 h, after which morphological and Gram stain examinations were conducted. Colonies of interest were selected for further inoculation onto sheep blood agar plates (Becton Dickinson Microbiology Systems, Cockeysville, Md.) at 37°C overnight. The coagulase test (Coagulase Plasma System; Difco) was used to identify *S. aureus*. Methicillin-susceptible *S. aureus* (MSSA) was preliminarily detected by its characteristic growth on mannitol salt agar and the absence of growth in the presence of oxacillin, while growth on both agar plates was presumed to indicate the presence of MRSA. All isolates were inoculated onto Mueller-Hinton agar (Becton Dickinson Microbiology Systems) containing 6 μg of oxacillin per ml and 4% NaCl to confirm methicillin resistance (29).

Antimicrobial susceptibility testing. All *S. aureus* isolates were tested for their susceptibilities to oxacillin, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, vancomycin, rifampin, tetracycline, ofloxacin, and gentamicin by the NCCLS agar disk diffusion method (28). The susceptibilities of the isolates to moxifloxacin were tested by incubation with Mueller-Hinton agar containing 2 μg of moxifloxacin per ml at 37°C for 24 h. Etest strips (PDM Epsilometer; AB Biodisk, Solna, Sweden) were used to confirm the isolates' resistance to moxifloxacin.

SCC*mec* typing by multiplex PCR. Multiplex PCR for SCC*mec* typing was performed by the methods of Oliveira and de Lencastre (31). The *mecA* gene and seven different loci (loci A to H) along the *mecA* gene cassette were selected for amplification by PCR. The primer sets specific for these loci were as follows: for type I-specific locus A, primers F5′-TTCGAGTTGCTGATGAAGAAGG-3′ and R5′-ATTTACCACAAGGACTACCAGC-3′; for type II-specific locus B, primers F5′ATTCATCTGCCATTGGTGATGC-3′ and R5′-CGAATGAAGT GAAAGAAAGTGG; for type II- and III-specific locus C, primers F5′-ATCA AGACTTGCATTCAGGC-3′ and R5′-GCGGTTTCAATTCACTTGTC; for type I-, II-, and IV-specific locus D, primers F5′-CATCCTATGATAGCTTGG TC-3′ and R5′-CTAAATCATAGCCATGACCG-3′; for type III-specific locus E, primers F5′-GTGATTGTTCGAGATATGTGG-3′ and R5′-CGCTTTATCT GTATCTATCGC-3′; for type III-specific locus F, primers F5′-TTCTTAAGTA CACGCTGAATCG-3′ and R5′-GTCACAGTAATTCCATCAATGC-3′; for nonspecific locus G, primers F5′-CAGGTCTCTTCAGATCTACG-3′ and R5′-GAGCCATAAACACCAATAGCC-3′; for nonspecific locus H, primers F5′-C AGGTCTCTTCAGATCTACG-3′ and R5′-GAAGAATGGGGAAAGCTTCA C-3′; and for the specific *mecA* gene, primers 5′-TCCAGATTACAACTTCAC CAGG-3′ and R5′-CCACTTCATATCTTGTAACG-3′.

Genomic fingerprinting by PFGE. Total DNA was prepared and pulsed-field gel electrophoresis (PFGE) was performed as described previously (22). The restriction enzyme SmaI was used at the temperature proposed by the manufacturer. The band patterns were visually compared and classified as indistinguishable (no differences), closely related (clonal variants, one to three band differences), possibly related (four to six band differences), and unrelated (more than six band differences) by the use of previously described criteria (38). Isolates with banding patterns that differed from the main pattern by up to three bands were considered to represent subtypes of the main type.

To identify PFGE polymorphisms, each sample was analyzed by using Molecular Analyst Fingerprinting, Fingerprinting Plus, and Fingerprinting DST software (Bio-Rad Laboratories, Richmond, Calif.). The grouping method was performed to deduce a dendrogram from the matrix by the unweighted pair group method with arithmetic averages clustering technique after calculation of similarities by using the Pearson correlation coefficient between each pair of organisms, and the PFGE patterns were distinguished at the 70% similarity level.

Questionnaire and statistical analysis. Each adult participant completed a standardized questionnaire. The questionnaires for the children were completed by their parents. The participant's age, gender, and medical history over the preceding 3 months, including previous hospitalization, medication history prior to receiving the screening test, and any underlying diseases, were correlated with the *S. aureus* colonization status. Chart reviews were conducted for the nursing home, hemodialysis, and hospitalized subjects.

Comparison of categorical variables and percentages between groups was done by the Pearson chi-square test or Fisher's exact test, as appropriate. Relative risk and 95% confidence intervals (CIs) were also calculated. Multivariate analysis was performed by using a stepwise logistic regression model. The threshold for a significant difference was designated a P value of <0.05. Factors associated with *S. aureus* or MRSA colonization with P values <0.05 were further studied by using a logistical regression model. All tests were two tailed.

RESULTS

Isolation of *S. aureus* from recruited subjects. A total of 2,231 subjects, including 1,838 community residents and 393 subjects from health care facility-related settings, were recruited for the study. Among the 538 of 2,231 (24.1%) subjects with *S. aureus* colonization, 91 (16.9%) were colonized with isolates which were resistant to oxacillin, yielding an MRSA colonization rate of 4.1% (91 of 2,231). The demographic and clinical characteristics and the rates of *S. aureus* and MRSA colonization for the 1,838 community subjects (851 community residents and 987 students) and 393 subjects in health care facility-related settings are shown in Table 1.

Comparison of the data for the 1,838 community residents and the 393 subjects recruited from health care facility-related settings revealed that the nasal *S. aureus* colonization rates of community residents (community subject *S. aureus* colonization [C-SA]) were significantly higher than those of subjects from health care facility-related settings (health care facility-related patient *S. aureus* colonization [H-SA]) (25.2 and 19.1%, respectively). On the contrary, the percentage of subjects colonized with C-MRSA isolates was significantly ($P = 0.002$) lower than the percentage of subjects colonized with H-MRSA isolates (3.5 and 6.9%, respectively). Subjects colonized with *S. aureus* had significantly higher MRSA colonization rates than health care facility-related subjects ($P < 0.001$) (Table 2).

Among the 1,838 community subjects, 463 (25.2%) had C-SA. Among these subjects, 314 were students and 149 were community residents. Sixty-four (13.8%) of 463 isolates were C-MRSA, including 33 from students and 31 from other residents, yielding a C-MRSA colonization rate in the community of 3.5%. The C-SA rate was significantly higher ($P < 0.001$) among students than among other community residents. By analysis of the rate of C-MRSA colonization among the subjects with C-SA, the rate of C-MRSA colonization and C-SA was significantly higher ($P = 0.003$) among other residents than among students. No significant difference in MRSA col-

TABLE 1. Demographics, medical information, and rates of *S. aureus* and MRSA colonization in the community and health care facility-related subject groups

Characteristics	Community subjects (n = 1,838)		Health care facility related subjects (n = 393)				Total
	Residents	Students	Hemodialysis	Chronic-care facility	Acute-care wards	Health care worker	
No. of subjects	851	987	85	100	69	139	2,231
Age (yr [range])	1–90	2–18	26–85	10–93	17–86	17–60	1–93
% Males/% females	39.7/60.3	40.4/59.6	44.7/55.3	60/40	50.7/49.3	4.3/95.7	39.3/60.7
% of subjects with:							
DM	3.4	0	20	28	4.3	0.7	3.5
Hypertension	5.8	0.1	30.6	53	5.8	1.4	6.1
Chronic liver disease	2.0	0.3	20	25	2.9	2.9	3.0
Renal disease	0.6	0	100	2	0	0	1.2
Pulmonary disease	0.5	0.2	0	45	1.4	1.4	2.4
Gastrointestinal disease	10.6	1.9	4.7	16	5.8	13.7	6.8
Nasal disease[a]	20.2	15.2	3.5	0	4.3	18.7	15.9
Recent surgery[b]	1.1	0.6	0	0	0	0.7	0.7
Recent admission[b]	1.2	0.9	0	100	100	1.4	8.5
Recent visits for OPD[c]	31.3	34.2	100	0	8.7	32.4	33.2
Recent medication[a]	30.0	29.3	100	99	98.6	26.6	37.3
Recent antibiotic uses[a]	2.2	0.7	0	3	7.2	10.8	2.2
S. aureus colonization	17.5	31.8	15.3	15	23.2	22.3	24.1
MRSA colonization	3.6	3.3	5.9	11	5.8	5	4.1

[a] Nasal diseases including allergic rhinitis, sinusitis, and any anatomic abnormality of the nose, including tumors.
[b] The medical history was traced to 3 months before surveillance testing.
[c] OPD, obstructive pulmonary disease.

onization rates was found between other residents and students (Table 2).

The H-MRSA rates ranged from 5 to 11% (average, 6.9%). The highest MRSA colonization rate was seen among subjects recruited from chronic-care facilities (11%). No significant differences in the rates of H-SA (P = 0.230) and H-MRSA colonization (P = 0.169) between patients from a chronic-care facility, a hemodialysis center, and acute-care wards and health care workers were observed. However, among the subjects with H-SA, the ratio of H-MRSA colonization/H-SA was significantly higher among patients than among health care workers (P = 0.042) (Table 2).

Risk factors for C-SA and C-MRSA colonization. Analysis of community cases revealed that age group ($P < 0.001$), the absence of diabetes mellitus (DM) (P = 0.022), and nasal illness (P = 0.05) (Table 3) were significantly associated with C-SA. Further analysis of these factors by use of a logistic regression procedure revealed that age and nasal illness were significantly related to nasal *S. aureus* colonization ($P < 0.001$ and P = 0.017, respectively). Subjects aged 0 to 10, 11 to 20, and 71 to 80 years had higher estimated probabilities of C-SA (37.6, 27.3, and 24.4%, respectively) than other age groups (18%). A relatively high frequency of C-SA was observed for subjects aged <10 years (95% CI, 0.328 to 0.425), 11 to 20 years (95% CI, 0.242 to 0.305), and 71 to 80 years (95% CI, 0.124 to 0.403).

Univariate analysis revealed that gastrointestinal diseases (P = 0.011; odds ratio [OR], 2.74; 95% CI, 1.32 to 5.70) and recent admission to a hospital (P = 0.026; OR, 5.401; 95% CI, 1.53 to 19.03) were significant risk factors for C-MRSA colonization. Recent medication (P = 0.09; OR, 1.55, 95% CI, 0.93 to 2,59) showed a trend toward being a major risk factor for C-MRSA colonization (Table 3). Logistic regression analysis of these factors revealed that recent hospital admission (P = 0.024) and recent gastrointestinal illness (P = 0.021) were significant risk factors for C-MRSA colonization.

TABLE 2. Rates of colonization with *S. aureus* and MRSA between the community and health care facility-related subjects

Colonizer	No. (%) of community subjects				Health care facility-related subjects						
					Patients (n = 254)						
	Residents (n = 851)	Student (n = 987)	Subtotal	P value[a]	Hemodialysis (n = 85)	Chronic-care facility (n = 100)	Acute-care wards (n = 69)	Health care workers (n = 139)	Subtotal	P value[b]	P value[c]
S. aureus	149 (17.5)	314 (31.8)	463 (25.2)	<0.001	13 (15.3)	15 (15)	16 (23.2)	31 (22.3)	75 (19.1)	0.230	0.010
MRSA	31 (3.6)	33 (3.3)	64 (3.5)	0.727	5 (5.9)	11 (11)	4 (5.8)	7 (5)	27 (6.9)	0.288	0.002
MRSA and *S. aureus*[d]	20.8	10.5	13.8	0.003	38.5	73.3	25	22.6	36	0.042	<0.001

[a] P value by comparison of residents and students among the community subjects.
[b] P value by comparison of patients and health care workers among health care facility-related subjects.
[c] P value by comparison of community and hospital-related subjects.
[d] Values for MRSA and colonization are in percent.

TABLE 3. Association of C-SA and C-MRSA with underlying diseases among community subjects

Underlying disease	No. of subjects		P value for C-SA vs non-C-SA[a]	No. of subjects		*P* value for C-MRSA vs non-C-MRSA
	C-SA (n = 463)	Non-C-SA (n = 1,375)		C-MRSA (n = 64)	Non-C-MRSA (n = 1,773)	
DM	2	27	0.022	0	29	0.302
Hypertension	10	40	0.391	1	49	0.562
Chronic liver disease	2	18	0.116	1	19	0.710
Renal disease	0	5	0.339	0	5	0.671
Pulmonary disease	1	5	1.000	0	6	0.641
Gastrointestinal disease	26	83	0.74	9	100	0.011
Nasal disease	95	227	0.05	14	308	0.352
Recent surgery	2	13	0.382	1	14	0.500
Recent admission	6	13	0.595	3	16	0.026
Recent OPD[b] visits	144	460	0.351	24	579	0.418
Recent medication	131	413	0.477	25	518	0.090
Recent antibiotics	3	23	0.106	1	25	0.605

[a] *P* value for significant difference among underlying diseases. Fisher's exact test instead of Pearson's chi-square test was performed when any expected count was less than 5 by statistical analysis.
[b] OPD, obstructive pulminary disease.

Antimicrobial susceptibility. All 538 *S. aureus* isolates were susceptible to vancomycin, while 91 (16.9%) isolates were MRSA. Resistance to erythromycin, clindamycin, tetracycline, and gentamicin was found in 55.8, 37.5, 60.4, and 19.5% of the isolates, respectively. Less than 5% of isolates were resistant to trimethoprim-sulfamethoxazole, rifampin, ofloxacin, and moxifloxacin. For C-MSSA and C-MRSA colonization isolates, the rates of resistance to erythromycin (48.1 and 90.6%, respectively), clindamycin (25.8 and 90.6%, respectively), trimethoprim-sulfamethoxazole (12.8 and 35.9%, respectively), tetracycline (53.1 and 95.3%, respectively), ofloxacin (1 and 12.5%, respectively), and gentamicin (8.8 and 64.1%, respectively) were significantly different (Table 4).

SCC*mec* gene typing of nasal colonization isolates and clinical isolates. In addition to 91 MRSA nasal colonization strains, 17 MRSA isolates from clinical specimens (11 from pus, 3 from sputum, 2 from blood, and 1 from urine) collected from hospitalized patients and matched by the area and the time period of collection were included in the molecular typing analysis. All of the MRSA isolates had the *mecA* gene. The results of SCC*mec* typing of the nasal colonization isolates from the subjects in the community, health care facility-related subjects, and clinical isolates from patients are shown in Table 5. A total of seven different SCC*mec* types were identified by multiplex PCR. In addition to previously described SCC*mec* types, two new variants were detected. Positive amplification of loci with locus-specific PCR primers revealed that one variant belonged to SCC*mec* type III, while the other appeared to be either a type I or a type III variant (Fig. 1). SCC*mec* type IV was the most common among the community colonizers (87.5%). The SCC*mec* type III group (type III, type IIIA, and type III variants) (51.85%) and SCC*mec* type IV (40.7%) were the two most common types among the health care facility-related colonization isolates. Among the clinical isolates from hospitalized patients, all except one belonged to the SCC*mec* type III group (10 isolates of type III, 4 isolates of type IIIA, and 2 isolates of the type III variant) (94.1%). Nine isolates were found to be a new type III variant. These isolates were amplified with *mecA*-specific locus E and F primer sets, which were specific for the type III *mecA* gene. One isolate was designated a new type variant, and multiplex PCR revealed

TABLE 4. Antimicrobial susceptibilities of MRSA isolates from community and hospital-related colonizers and hospitalized patints

Drug	% Resistant			*P* value[a]
	Community colonizers (n = 64)	Hospital-related colonizers (n = 27)	Clinical hospital strains (n = 17)	
Erythromycin	90.6	96.3	100	0.231
Clindamycin	90.6	96.3	100	0.231
TMP-SMX[b]	35.9	55.6	94.1	<0.0001
Vancomycin	0	0	0	
Rifampin	3.1	29.6	41.2	0.0002
Tetracycline	95.3	85.2	100	0.4883
Ofloxacin	12.5	55.6	94.1	<0.0001
Gentamicin	64.1	88.9	82.4	0.1248
Moxifloxacin	1.6	33.3	41.2	<0.0001

[a] *P* value by Fisher's exact test for the significant difference in drug resistance among community colonizers and clinical hospital strains.
[b] TMP-SMX, trimethoprim-sulfamethoxazole.

TABLE 5. SCC*mec* types of MRSA isolates from community and hospital-related colonized subjects and from hospitalized patients

SCC*mec* type	No. of isolates		
	Community colonizers (n = 64)	Hospital-related colonizers (n = 27)	Clinical hospital strains (n = 17)
I	1	1	0
II	0	1	0
III	1	9	10
IIIA	0	2	4
IV	56	11	1
Type III variant[a]	4	3	2
Not typed[b]	1	0	0
New variant[c]	1	0	0

[a] The new type III variant was defined when specific *mecA* type III loci E and F were present (31).
[b] One isolate that was not typed was positive only for the *mecA* gene, with no PCR product obtained with the SCC*mec* gene tested.
[c] An isolate contained the specific A, D, E, and F loci, which were type I or type III specific and was designated the new type variant.

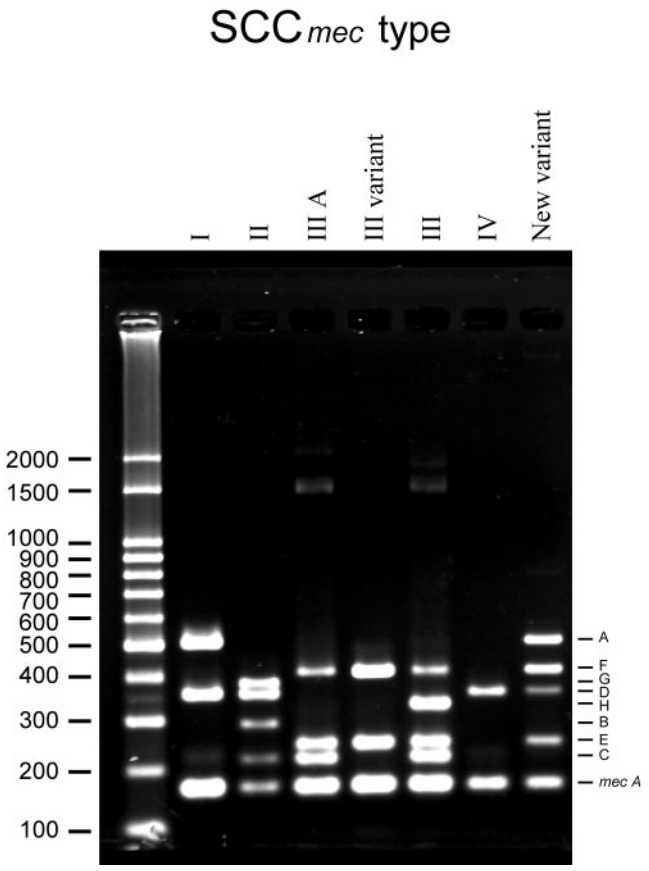

FIG. 1. Identification of SCC*mec* types by multiplex PCR. The lane on the left contains a molecular weight marker. Loci specific to a SCC*mec* type by PCR were as follows: A, type I; B, type II; C, types II and III; D, types I, II, and IV, E, type III; F, type III; G and H, nonspecific for a SCC*mec* type.

that this isolate contained the A, D, E, and F loci, which were either type I or type III specific.

PFGE analysis of C-MRSA and H-MRSA isolates. A wide diversity of pulsotypes was found among the isolates from the different subject populations, as shown in Fig. 2. Six clusters that included 51 (47.2%) isolates were distinguished at the 70% similarity level, and their band patterns showed that they differed from each other by less than three bands. Except for one isolate from a community resident (isolate A27), all isolates of MRSA cluster I were from health care facility-related subjects or hospitalized patients. Nine (52.9%) of the 17 MRSA clinical hospital isolates from cluster VI or I were from patients at Pingtong Hospital. The isolates in clusters II, III, and V were all community isolates, indicating that C-MRSA isolates were easily distinguishable from the clinical hospital strains and the health care facility-related isolates. Clusters I and VI comprised community, health care facility-related, and clinical hospital isolates, which indicates that some of the isolates colonizing community and health care facility-related subjects were clonally related to clinical hospital isolates.

Colonization with *S. aureus* and MRSA among subjects without health care facility-related risk factors. Subjects from the community and health care-related settings were stratified by their health care-related predisposing factors, including receiving medical service within the previous 3 months and being a health care worker. There were 1,025 subjects who were not health care professionals and who had not recently received health care services or any medication. *S. aureus* was isolated from 277 of these subjects. Thirty-five MRSA nasal isolates were found, indicating a 3.4% MRSA colonization rate among community residents without risk factors. Other subjects who had any health care-related predisposing risk factor or who were health care workers had an MRSA colonization rate of 4.6% (56 of 1,206 subjects) ($P = 0.144$).

The rates of drug resistance were significantly lower among MRSA isolates from subjects without risk factors than among isolates from subjects who had any predisposing risk factor or who were health care workers, as follows: rifampin, 2.4 and 18.8%, respectively; ofloxacin, 9.5 and 30.4%, respectively; and moxifloxacin, 2.4 and 17.4%, respectively. For the other antimicrobial agents tested, no differences in resistance rates were found between subjects with and without risk factors (rates of resistance to clindamycin, 92.9 and 82.6%, respectively; rates of resistance to erythromycin, 88.1 and 92.8%, respectively; rates of resistance to tetracycline, 95.2% and 89.9%, respectively; rates of resistance to gentamicin, 64.3% and 71.0%, respectively).

DISCUSSION

Community-acquired MRSA infections have raised concerns worldwide (10, 16, 18, 34). MRSA colonization may subsequently cause infections (7, 17, 40). Determination of whether community MRSA colonization originated from health care setting-related cases or by spread through horizontal transmission within the community may influence how this problem will be addressed.

In this study, subjects whose activities involved contact with a health care facility had a significantly higher rate of MRSA colonization than community subjects, even though the health care-facility related subjects had a lower overall *S. aureus* colonization rate than their community counterparts. Age was found to be the most significant factor for *S. aureus* colonization in this study. Recent admission to a hospital and gastrointestinal diseases were the most important factors associated with MRSA colonization among community subjects. The finding that recent admission was the major factor associated with MRSA colonization in community subjects is consistent with the findings of previous reports suggesting that C-MRSA might originate from contact with a hospital environment (6, 8, 36) but is contrary to the findings of an earlier report (26) that did not show any significant risk factors differentiating patients with C-MRSA and MSSA infections.

The rate of resistance to all the antimicrobials tested except vancomycin among our MRSA nasal isolates (over 10%) was higher than that in a previous study of urban poor individuals from San Francisco, where erythromycin and ciprofloxacin were the only two drugs to which rates of resistance were >10% (6). The rates of multiple-drug resistance among our MRSA isolates were also higher than those presented in other reports (3, 9, 21), in which most clinical C-MRSA isolates were susceptible to various antibiotics except beta-lactams. The rate of resistance to clindamycin (92.9%) among the C-MRSA iso-

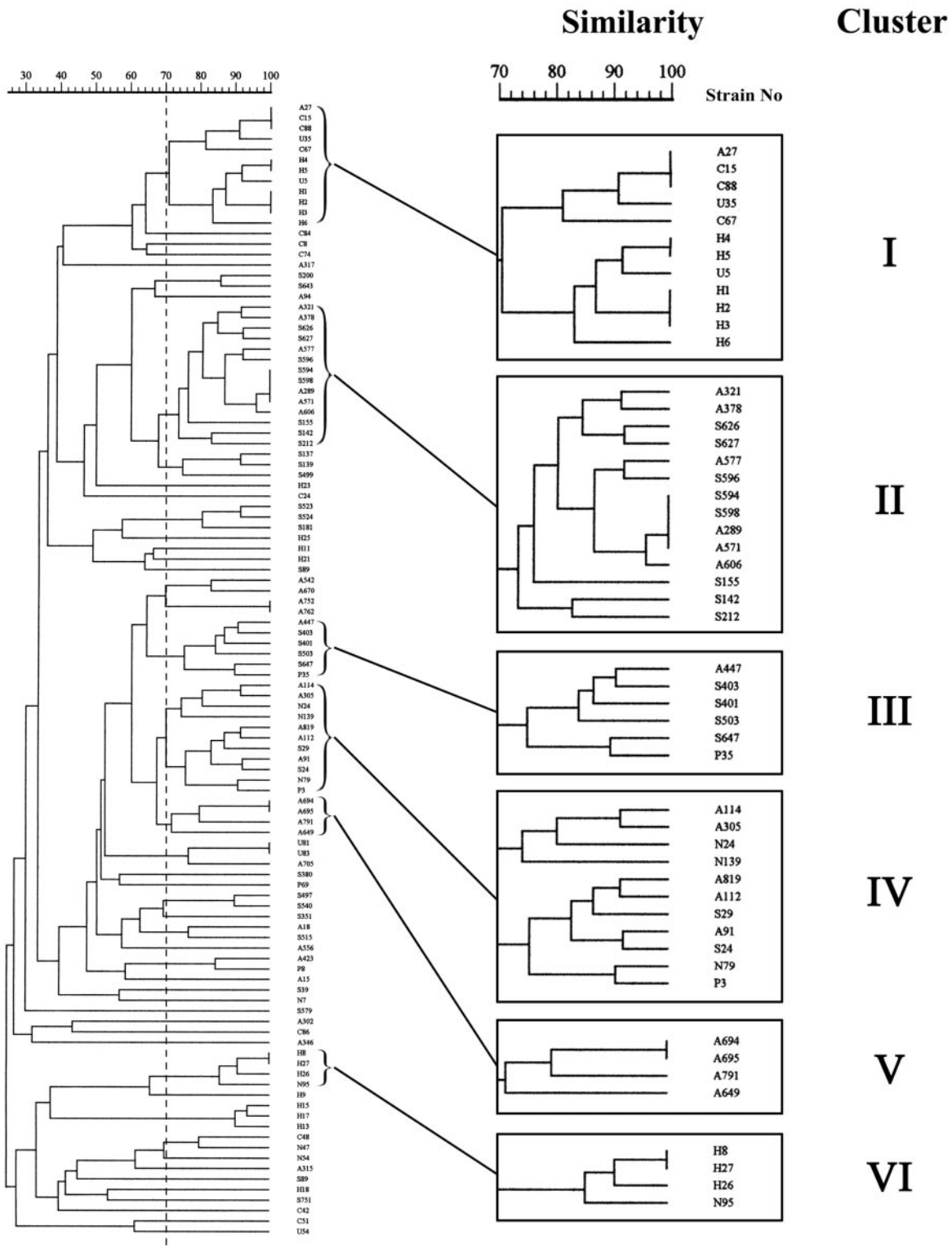

FIG. 2. PFGE patterns of 91 nares-colonizing MRSA isolates and 17 clinical MRSA isolates from a regional hospital. Similarities >70% represent the clonal spread of strains. The first letter of each isolate designation indicates the origin of the isolate, as follows: colonization isolates from community residents (A), students (S), patients in chronic-care facilities (C), patients in acute-care wards (P), health care workers (N) and hemodialysis patients (U) and clinical isolates from infected hospitalized patients (H).

lates from subjects without risk factors in this study was also higher than that in a study from the United States (10), in which most C-MRSA isolates from subjects without risk factors were susceptible to clindamycin. The high rate of resistance to clindamycin among our community MRSA isolates (90.6%) was similar to the rate of resistance among clinical MRSA isolates in Taiwan (94.2%) (13, 41), indicating that clindamycin resistance is quite common among community and health care facility-related MRSA isolates in Taiwan.

This study found that recent receipt of medical services was the major factor associated with MRSA colonization as well as the high level of multiple-drug resistance in MRSA nasal isolates. These findings may be explained by the high rate of antibiotic use in the Taiwan community, as shown in a previous study (20) in which antimicrobial activity in urine was detected in 55.2% subjects on arrival at an emergency department and in 7.6% of high school students. Another study found that the proportion of patient visits resulting in antimicrobial therapy in primary care units was 13.4% in Taiwan and that 31.3% of patients with a diagnosis of the common cold received antibiotic treatment (5). These findings are indicative of the presence of strong selective pressure from antimicrobial use in the community.

We identified four factors which support the occurrence of transmission of MRSA outside the hospital setting. First, there was a high rate (3.41%) of MRSA colonization among community residents who did not have health care setting-related predisposing factors. Second, MRSA isolates from community residents with colonization had antibiograms which were different from those of the clinical hospital strains with regard to trimethoprim-sulfamethoxazole, rifampin, ofloxacin, and moxifloxacin resistance. Third, most of the MRSA isolates responsible for colonization of community subjects were of SCC*mec* type IV, whereas most of the clinical hospital strains were of SCC*mec* type III. Fourth, molecular typing of MRSA isolates by PFGE revealed that three clusters of MRSA isolates were mainly from colonized community residents, and no clinical hospital MRSA isolates were in the clusters mainly formed by colonizing community strains. This result suggests that the transmission of MRSA from the clinical setting to the community did not comprise the main source of MRSA colonization in the community.

With regard to the significant difference in trimethoprim-sulfamethoxazole, rifampin, ofloxacin, and moxifloxacin resistance between community colonizers and clinical hospital strains, the MRSA isolates from health care facility-related colonizers had antimicrobial resistance rates between the rates for the other two groups (Table 5). High proportions of the MRSA isolates from health care facility-related colonizers were of SCC*mec* type IV and type III, and these were predominantly community colonizing strains and clinical hospital strains, respectively. The mixed characteristics of the health care facility-related colonizing strains and PFGE clusters I, III, and IV among both community and heath care facility-related isolates suggest that MRSA colonization among health care facility-related subjects may be a route of transmission from hospitals to the community.

The risk factor assessment used in this study had several limitations. The proportion of study participants with recent antibiotic usage might have been underestimated because many patients might not have recognized that they had taken antibiotics, especially when the drugs were prescribed in a clinic as opposed to a hospital. The tracing of a 3-month medical history in this study was intended to limit the recall bias that might result from the review of a longer period. However relevant, data on the medical history from more than 3 months earlier could have been neglected, and these data may have been relevant, as MRSA colonization can continue for several years (2). Dermatological factors, like atopic eczema, which have been reported to be associated with MRSA carriage (39) were not considered in this study. Another possible risk factor for MRSA carriage not considered was the possibility that household members or friends of the study subjects may have been health care workers or may have had chronic diseases requiring frequent hospital visits. Heterogeneous drug resistance (heteroresistance) might not have been effectively detected by our method (4, 25), and a small percentage of isolates that carry the *mecA* gene are phenotypically susceptible to methicillin, which may have resulted in underestimation of the rate of MRSA colonization in the community. Nevertheless, the rate of MRSA colonization was found to be higher in this community surveillance study than in recent studies in other regions (6, 33, 36).

The presence of penicillinase-producing *S. aureus* strains in hospitals in the early 1950s was followed by a high prevalence of penicillin resistance among hospital and community strains in the 1970s (15). The high prevalence of MRSA in hospitals and recent increases in reports of community MRSA infections without traditional health care facility-related risk factors (10) suggest that a similar transfer of drug resistance from hospitals to the community may occur soon in Taiwan. Regardless of the impact of the health care facility-related cases, our findings that 3.5% of the community population was colonized with MRSA and that 3.4% of MRSA-colonized subjects had no predisposing risk factors indicate that the burden of MRSA in the community is heavy in Taiwan. The MRSA isolates examined in this study showed high rates of resistance to most antistaphylococcal agents, reflecting the difficulty in providing effective antimicrobial therapy if infections due to such resistant pathogens were to occur. The findings that the health care setting-related group had a higher rate of MRSA colonization than the community group and that MRSA colonization was related to recent hospitalization indicate the need for education and infection control measures for health care workers, patients returning from hospitals to the community, and individuals receiving medical treatment. Such measures may reduce the levels of transmission of MRSA from health care settings to the community. The implications of MRSA colonization, infection, and treatment should be explained to the patient and close relatives who assist with the patient's bodily care. Although routes of MRSA transmission from the hospital setting to the community exist, the molecular evidence of the presence of colonizing strains in the community and the high rate of MRSA colonization among people without a relationship to the hospital setting suggest that further measures to control antibiotic usage to reduce selective pressure for antibiotic resistance are urgently needed in the community as well as in hospitals.

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REFERENCES

1. **Aires de Sousa, M., M. I. Crisostomo, I. S. Sanches, J. S. Wu, J. Fuzhong, A. Tomasz, and H. de Lencastre.** 2003. Frequent recovery of a single clonal type of multidrug-resistant *Staphylococcus aureus* from patients in two hospitals in Taiwan and China. J. Clin. Microbiol. **41:**159–163.
2. **Boyce, J. M.** 1998. Are the epidemiology and microbiology of methicillin-resistant *Staphylococcus aureus* changing? JAMA **279:**623–624.
3. **Centers for Disease Control and Prevention.** 1999. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*—Minnesota and North Dakota, 1997–1999. JAMA **282:**1123–1125.
4. **Chambers, H. F., and M. Sachdeva.** 1990. Binding of beta-lactam antibiotics to penicillin-binding proteins in methicillin-resistant *Staphylococcus aureus.* J. Infect. Dis. **161:**1170–1176.
5. **Chang, S. C., M. N. Shiu, and T. J. Chen.** 2001. Antibiotic usage in primary care units in Taiwan after the institution of national health insurance. Diagn. Microbiol. Infect. Dis. **40:**137–143.
6. **Charlebois, E. D., D. R. Bangsberg, N. J. Moss, M. R. Moore, A. R. Moss, H. F. Chambers, and F. Perdreau-Remington.** 2002. Population-based community prevalence of methicillin-resistant *Staphylococcus aureus* in the urban poor of San Francisco. Clin. Infect. Dis. **34:**425–433.
7. **Cole, A. M., S. Tahk, A. Oren, D. Yoshioka, Y. H. Kim, A. Park, and T. Ganz.** 2001. Determinants of *Staphylococcus aureus* nasal carriage. Clin. Diagn. Lab. Immunol. **8:**1064–1069.
8. **Goetz, A., K. Posey, J. Fleming, S. Jacobs, L. Boody, M. M. Wagener, and R. R. Muder.** 1999. Methicillin-resistant *Staphylococcus aureus* in the community: a hospital-based study. Infect. Control Hosp. Epidemiol. **20:**689–691.
9. **Gross-Schulman, S., D. Dassey, L. Mascola, and C. Anaya.** 1998. Community-acquired methicillin-resistant *Staphylococcus aureus.* JAMA **280:**421–422.
10. **Herold, B. C., L. C. Immergluck, M. C. Maranan, D. S. Lauderdale, R. E. Gaskin, S. Boyle-Vavra, C. D. Leitch, and R. S. Daum.** 1998. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA **279:**593–598.
11. **Hiramatsu, K., L. Cui, M. Kuroda, and T. Ito.** 2001. The emergence and evolution of methicillin-resistant *Staphylococcus aureus.* Trends Microbiol. **9:**486–493.
12. **Ho, M., L. C. McDonald, T. L. Lauderdale, L. L. Yeh, P. C. Chen, and Y. R. Shiau.** 1999. Surveillance of antibiotic resistance in Taiwan, 1998. J. Microbiol. Immunol. Infect. **32:**239–249.
13. **Hsueh, P. R., C. Y. Liu, and K. T. Luh.** 2002. Current status of antimicrobial resistance in Taiwan. Emerg. Infect. Dis. **8:**132–137.
14. **Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, C. Tiensasitorn, and K. Hiramatsu.** 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus.* Antimicrob. Agents Chemother. **45:**1323–1336.
15. **Jessen, O., K. Rosendal, P. Bulow, V. Faber, and K. R. Eriksen.** 1969. Changing staphylococci and staphylococcal infections. A ten-year study of bacteria and cases of bacteremia. N. Engl. J. Med. **281:**627–635.
16. **Jones, T. F., M. E. Kellum, S. S. Porter, M. Bell, and W. Schaffner.** 2002. An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus.* Emerg. Infect. Dis. **8:**82–84.
17. **Kluytmans, J., A. van Belkum, and H. Verbrugh.** 1997. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin. Microbiol. Rev. **10:**505–520.
18. **Layton, M. C., W. J. Hierholzer, Jr., and J. E. Patterson.** 1995. The evolving epidemiology of methicillin-resistant *Staphylococcus aureus* at a university hospital. Infect. Control Hosp. Epidemiol. **16:**12–17.
19. **Lin, J. C., J. S. Wu, and F. Y. Chang.** 2000. Community-acquired methicillin-resistant *Staphylococcus aureus* endocarditis with septic embolism of popliteal artery: a case report. J. Microbiol. Immunol. Infect. **33:**57–59.
20. **Liu, Y. C., W. K. Huang, T. S. Huang, and C. M. Kunin.** 1999. Extent of antibiotic use in Taiwan shown by antimicrobial activity in urine. Lancet **354:**1360.
21. **Ma, X. X., T. Ito, C. Tiensasitorn, M. Jamklang, P. Chongtrakool, S. Boyle-Vavra, R. S. Daum, and K. Hiramatsu.** 2002. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **46:**1147–1152.
22. **Macfarlane, L., J. Walker, R. Borrow, B. A. Oppenheim, and A. J. Fox.** 1999. Improved recognition of MRSA case clusters by the application of molecular subtyping using pulsed-field gel electrophoresis. J. Hosp. Infect. **41:**29–37.
23. **Madani, T. A., N. A. Al Abdullah, A. A. Al Sanousi, T. M. Ghabrah, S. Z. Afandi, and H. A. Bajunid.** 2001. Methicillin-resistant *Staphylococcus aureus* in two tertiary-care centers in Jeddah, Saudi Arabia. Infect. Control Hosp. Epidemiol. **22:**211–216.
24. **Maguire, G. P., A. D. Arthur, P. J. Boustead, B. Dwyer, and B. J. Currie.** 1998. Clinical experience and outcomes of community-acquired and nosocomial methicillin-resistant *Staphylococcus aureus* in a northern Australian hospital. J. Hosp. Infect. **38:**273–281.
25. **Maranan, M. C., B. Moreira, S. Boyle-Vavra, and R. S. Daum.** 1997. Antimicrobial resistance in staphylococci. Epidemiology, molecular mechanisms, and clinical relevance. Infect. Dis. Clin. N. Am. **11:**813–849.
26. **Moreno, F., C. Crisp, J. H. Jorgensen, and J. E. Patterson.** 1995. Methicillin-resistant *Staphylococcus aureus* as a community organism. Clin. Infect. Dis. **21:**1308–1312.
27. **Naimi, T. S., K. H. LeDell, D. J. Boxrud, A. V. Groom, C. D. Steward, S. K. Johnson, J. M. Besser, C. O'Boyle, R. N. Danila, J. E. Cheek, M. T. Osterholm, K. A. Moore, and K. E. Smith.** 2001. Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996–1998. Clin. Infect. Dis. **33:**990–996.
28. **National Committee for Clinical Laboratory Standards.** 2002. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
29. **National Committee for Clinical Laboratory Standards.** 2002. Performance standards for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
30. **Okuma, K., K. Iwakawa, J. D. Turnidge, W. B. Grubb, J. M. Bell, F. G. O'Brien, G. W. Coombs, J. W. Pearman, F. C. Tenover, M. Kapi, C. Tiensasitorn, T. Ito, and K. Hiramatsu.** 2002. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J. Clin. Microbiol. **40:**4289–4294.
31. **Oliveira, D. C., and H. de Lencastre.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus.* Antimicrob. Agents Chemother. **46:**2155–2161.
32. **Rings, T., R. Findlay, and S. Lang.** 1998. Ethnicity and methicillin-resistant *S. aureus* in South Auckland. N. Z. Med. J. **111:**151.
33. **Sa-Leao, R., I. S. Sanches, I. Couto, C. R. Alves, and H. de Lencastre.** 2001. Low prevalence of methicillin-resistant strains among *Staphylococcus aureus* colonizing young and healthy members of the community in Portugal. Microb. Drug Resist. **7:**237–245.
34. **Salmenlinna, S., O. Lyytikainen, and J. Vuopio-Varkila.** 2002. Community-acquired methicillin-resistant *Staphylococcus aureus*, Finland. Emerg. Infect. Dis. **8:**602–607.
35. **Shahin, R., I. L. Johnson, F. Jamieson, A. McGeer, J. Tolkin, E. L. Ford-Jones, et al.** 1999. Methicillin-resistant *Staphylococcus aureus* carriage in a child care center following a case of disease. Arch. Pediatr. Adolesc. Med. **153:**864–868.
36. **Shopsin, B., B. Mathema, J. Martinez, E. Ha, M. L. Campo, A. Fierman, K. Krasinski, J. Kornblum, P. Alcabes, M. Waddington, M. Riehman, and B. N. Kreiswirth.** 2000. Prevalence of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in the community. J. Infect. Dis. **182:**359–362.
37. **Stacey, A. R., K. E. Endersby, P. C. Chan, and R. R. Marples.** 1998. An outbreak of methicillin resistant *Staphylococcus aureus* infection in a rugby football team. Br. J. Sports Med. **32:**153–154.
38. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
39. **Trividic, M., M. L. Gauthier, A. Sparsa, M. C. Ploy, M. Mounier, S. Boulinguez, C. Bedane, and J. M. Bonnetblanc.** 2002. Methi-resistant *Staphylococcus aureus* in dermatological practice: origin, risk factors and outcome. Ann. Dermatol. Venereol. **129:**27–29. (In French.)
40. **von Eiff, C., K. Becker, K. Machka, H. Stammer, G. Peters, et al.** 2001. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. N. Engl. J. Med. **344:**11–16.
41. **Wang, J. T., Y. C. Chen, T. L. Yang, and S. C. Chang.** 2002. Molecular epidemiology and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* in Taiwan. Diagn. Microbiol. Infect. Dis. **42:**199–203.